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Semi-national surveillance of fungaemia in Denmark 2004–2006: increasing incidence of fungaemia and numbers of isolates with reduced azole susceptibility

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ABSTRACT

A semi-national laboratory-based surveillance programme for fungaemia was initiated in 2003 that now covers *c.* 3.5 million inhabitants (64%) of the Danish population. In total, 1089 episodes of fungaemia were recorded during 2004–2006, corresponding to an annual incidence of 10.4/100 000 inhabitants. The annual number of episodes increased by 17% during the study period. *Candida* spp. accounted for 98% of the fungal pathogens. Although *Candida albicans* remained predominant, the proportion of *C. albicans* decreased from 66.1% in 2004 to 53.8% in 2006 ($p < 0.01$), and varied considerably among participating departments, e.g., from 51.1% at a university hospital in Copenhagen to 67.6% in North Jutland County. *Candida glabrata* ranked second, and increased in proportion from 16.7% to 22.7% (p 0.04). *Candida krusei* was isolated rarely (4.1%), but the proportion doubled during the study period from 3.2% to 6.4% (p 0.06). MIC distributions of amphotericin B and caspofungin were in close agreement with the patterns predicted by species identification; however, decreased susceptibility to voriconazole, defined as an MIC of >1 mg/L, was detected in one (2.5%) *C. glabrata* isolate in 2004 and in 12 (14.0%) isolates in 2006 (p 0.03). Overall, the proportion of isolates with decreased susceptibility to fluconazole exceeded 30% in 2006. The incidence of fungaemia in Denmark was three-fold higher than that reported from other Nordic countries and is increasing. Decreased susceptibility to fluconazole is frequent, and a new trend towards *C. glabrata* isolates with elevated voriconazole MICs was observed.

Keywords *Candida* spp., Denmark, fluconazole, fungaemia, resistance, surveillance

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INTRODUCTION

A semi-national surveillance programme for fungaemia was initiated in Denmark in 2003, with a notably high incidence of 11 episodes/100 000 inhabitants being recorded during the first year [1]. With this background, the surveillance scheme was continued and extended to cover 64% of the Danish population. In three other Nordic countries, the incidence of candidaemia has been in the range of 2.2–3.5/100 000, with 67–70% of cases being caused by *Candida albicans* [2–4]. In European surveys, incidences of 2.5–5.0/100 000 have been reported [5–8], with 56% (43–67%) of the cases in a recent European study covering France, Germany, Austria, Spain, Sweden and the UK being caused by *C. albicans* [9], while incidences of 6.0–8.7/100 000 have been reported in most population-based studies in the USA [10,11]. One major exception is a study that reported an incidence of 7.1/100 000 in Connecticut and 24/100 000 in Baltimore, despite the fact that the incidences calculated in terms of number of discharges were comparable [12].

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C. albicans remains the predominant species on both sides of the Atlantic Ocean, with frequencies varying from *c.* 50% in North America and Spain to *c.* 70% in recent studies from Norway and Finland [2,4,13]. The proportion of *Candida glabrata* isolates has remained constant overall, at 9.5–12.0% according to the ARTEMIS DISK surveillance programme for 1997–2003, which now includes >130 000 isolates from 39 countries [14]. Geographical differences exist, with a higher proportion of *C. glabrata* isolates in the USA (*c.* 20%) than in most other countries (4–14%) [2,3,9,15,16]. In this context, the first years of surveillance in Denmark revealed an unexpectedly high proportion of *C. glabrata* (20%) [1]. The present article describes the results from the first 3 years of the continued programme (2004–2006), which now covers two-thirds of the Danish population, thereby providing the most comprehensive study of fungaemia in Denmark to date.

MATERIALS AND METHODS

Surveillance and population

During the 3-year period from January 2004 to December 2006, fungal blood isolates were collected at the eight major Danish departments of clinical microbiology at Rigshospitalet and Hvidovre Hospital (serving Copenhagen City hospitals and the island of Bornholm since 1 January 2005), Herlev Hospital (serving hospitals in the county of Copenhagen), Statens Serum Institut (serving hospitals in Bornholm during the period 1 January 2004 to 31 December 2004 and the county of Roskilde), Hillerød Hospital (serving hospitals in the county of Frederiksborg), Odense University Hospital (serving hospitals in the county of Funen), Skejby Hospital (serving hospitals in the county of Aarhus), and Aalborg Hospital (serving hospitals in the county of North Jutland). Together, the eight departments served 13 university and 28 district hospitals in the municipality of Copenhagen and the respective counties, with a total of 11 033 hospital beds (67.8% of the total non-psychiatric hospital beds in Denmark). In total, these hospitals served a total population of 3 490 465–3 509 803 (2004–2006) or 64.5% of the Danish population. In addition, the university hospitals were secondary- or tertiary-care centres for the entire country. In particular, all solid-organ transplantations and autologous bone marrow transplantations in Denmark were performed at the participating hospitals, and all allogenic bone marrow transplantations and liver transplantations were performed at Rigshospitalet. However, it was not feasible to analyse the referred cases separately. The characteristics of the eight participating departments of clinical microbiology are summarised in Table 1. Information concerning the number of non-psychiatric admissions was retrieved from http://www.sundhedsdata.sst.dk.

During the study period, four departments used the BacT/ALERT (bioMérieux, Marcy l'Etoile, France) blood culture system and four used the BACTEC (Becton Dickinson, Franklin Lakes, NJ, USA) blood culture system. Information concerning the total number of bloodstream isolates was retrieved from each department's laboratory information system. Successive blood cultures that yielded fungi from the same patient were considered to be distinct episodes if they occurred ≥21 days apart or were caused by different species. Most (90.5%) of the isolates were sent to the reference laboratory (Unit of Mycology and Parasitology, Statens Serum Institut, Copenhagen, Denmark) for verification of species identification and susceptibility testing, but 108 (9.5%) isolates were not referred and no susceptibility data for these isolates were available for analysis. In addition, voriconazole susceptibility testing was not performed for 74 (6.5%) isolates collected in January–March 2004, as this was not commenced until 1 April 2004.

Species identification

Species identification at the reference laboratory was based on colony morphology on chromogenic agar (CHROMagar, Paris, France), microscopic morphology on corn-meal agar and rice plus Tween agar (SSI Diagnostika, Hillerød, Denmark), growth at 35°C and 43°C, and results obtained using the ATB ID32C system (bioMérieux). In addition, rapid commercial tests for the identification of *Candida dubliniensis* and *C. glabrata* were used increasingly during the study period (BICHRO-DUBLI and Glabrata RTT; Fumouze Diagnostics, Simoco, Denmark).

Susceptibility testing

Susceptibility testing was performed according to EUCAST discussion document E7.1 [17]. Manufacturers and stock solutions were as follows: dimethylsulphoxide (DMSO) (Sigma-Aldrich, Vallensbaek Strand, Denmark); fluconazole (Pfizer, Ballerup, Denmark; 10 000 mg/L); amphotericin B (Sigma-Aldrich; 5000 mg/L in DMSO); caspofungin (Merck, Sharp and Dohme, Glostrup, Denmark; 5000 mg/L in DMSO); itraconazole (Janssen-Cilag, Birkerød, Denmark; 5000 mg/L in DMSO); and voriconazole (Pfizer; 5000 mg/L in DMSO). Two-fold dilutions in RPMI medium with glucose 2% w/v were prepared in microtitre plates and stored at −20°C until use. Microtitre plates were read spectrophotometrically at 490 nm. The MIC was defined as the lowest drug concentration giving 100% growth inhibition for amphotericin B or 50% growth inhibition for the other compounds. *Candida krusei* ATCC 6862 was included as a control strain in each run; the MIC determinations were accepted if they were within the published ranges for amphotericin B, fluconazole, itraconazole and voriconazole [18], and within 0.25–2 mg/L for caspofungin (for which no quality control range has yet been published).

Consumption of antifungal compounds

Information concerning consumption of antifungal agents in defined daily doses (DDD) was available from the Danish Medicines Agency and from hospital pharmacies serving the participating university hospitals.

Statistical analysis

The infection rate was calculated by dividing annual numbers of distinct episodes by either catchment population and multiplying by 10^5, or by the numbers of non-psychiatric

Table 1. Characteristics of the eight participating centres showing the rates of fungaemia and species distribution of isolates recovered in the 3-year period 2004–2006

	Funen County	Roskilde County and Bornholm[a]	Copenhagen County	Rigshospitalet	North Jutland County	Aarhus County	Frederiksborg County	Copenhagen City hospitals and Bornholm[a]	Total
Characteristics of participating centres									
No. of hospitals (university/district)	9 (1/8)	3 (1/2)/2 (1/1)[a]	4 (3/1)	1 (1/0)	7 (1/6)	8 (3/5)	4 (0/4)	5 (1/4)/6 (1/5)[a]	41 (11/30)
Population served in the catchment area[b]	476 604	282 616/239 199[a]	618 337	65 169	495 222	657 433	375 988	523 445/566 862[a]	3 499 645
No. of admissions in 2005	94 905	49 420	135 352	65 169	97 322	133 361	80 491	119 817	733 996
Blood culture system	BACTEC	BACTEC	BACTEC	BACTEC	BacT/ALERT	BacT/ALERT	BacT/ALERT	BacT/ALERT	
No. of bottles/blood culture (days of culture)	2 (6)	3 (5)	4 (5)	2 (7)	3 (7)	2 (6)	4 (5–6)	4 (5)	
Rate of fungaemia									
Isolates/100 000 inhabitants	11.8	7.9	7.0	NA[d]	9.6	9.7	7.7	7.8	10.8
Isolates/1000 admissions[c]	0.59	0.36	0.32	1.125	0.49	0.48	0.36	0.39	0.51
Species distribution									
Candida albicans	65.7% (111)	59.6% (34)	65.4% (85)	54.5% (122)	67.6% (96)	59.7% (114)	55.2% (48)	51.1% (68)	59.8% (678)
Candida dubliniensis	3.0% (5)	3.5% (2)	2.3% (3)	2.2% (5)	2.1% (3)	2.1% (4)	2.3% (2)	3.8% (5)	2.6% (29)
Candida glabrata	11.2% (19)	17.5% (10)	17.8% (23)	20.1% (45)	17.6% (25)	21.5% (41)	27.6% (24)	33.8% (45)	20.5% (232)
Candida krusei	3.6% (6)	5.2% (3)	2.3% (3)	8.5% (19)	2.1% (3)	4.2% (8)	2.3% (2)	2.3% (3)	4.1% (47)
Candida parapsilosis	7.1% (12)	3.5% (2)	4.6% (6)	4.0% (9)	1.4% (2)	4.2% (8)	4.6% (4)	1.5% (2)	4.0% (45)
Candida tropicalis	4.7% (8)	7.0% (4)	3.8% (5)	4.9% (11)	2.1% (3)	5.2% (10)	4.6% (4)	5.3% (7)	4.6% (52)
Candida spp.	1.8% (3)	1.8% (1)	3.1% (4)	2.2% (5)	5.6% (8)	2.1% (4)	3.4% (3)	0.8% (1)	2.6% (29)
Non-*Candida* fungi[e]	3.0% (5)	1.8% (1)	0.8% (1)	3.6% (8)	1.4% (2)	1.7% (2)	0% (0)	1.5% (2)	1.9% (21)
Total	169	57	130	224	142	191	87	133	1133

[a]Blood cultures from the island of Bornholm were examined at Statens Serum Institute, also serving Roskilde County, in the period 1 January 2004 to 31 December 2004, and at Hvidovre (serving Copenhagen City hospitals) in the period 1 January 2005–31 December 2006. Figures are given as 2004/2005–2006 numbers.
[b]Mean number of inhabitants during the 3-year period.
[c]Based on number of somatic admissions during 2005.
[d]Not applicable, as Rigshospitalet has an extensive number of patients referred from other parts of Denmark because of the tertiary function of this hospital, which strongly affects the rate calculated in terms of number/100 000.
[e]Non-*Candida* fungi included nine *Saccharomyces cerevisiae*, four *Cryptococcus neoformans*, two *Fusarium solani*, two *Fusarium* spp., and one each of *Rhodotorula* spp., *Trichosporon inkin* and *Trichosporon mucoides*, as well as one mould that was not referred to the reference laboratory for species identification.

admissions and multiplying by 10^3. The strict definition of an episode meant that the infection rate was close to the true incidence, and therefore the two terms are used interchangeably. Fisher's exact test was used for comparison of changes in species distribution, and a chi-square test for trend was used for comparing the proportion of *C. glabrata* isolates for which the voriconazole MIC was >1 mg/L. p values <0.05 were considered to be statistically significant.

RESULTS

Epidemiology

During the 3-year period from 2004 to 2006, 1089 episodes of fungaemia were registered for 1040 patients. The numbers of patients (324, 339, 377), episodes (334, 363, 392) and isolates recovered (342, 382, 409) increased by 16.4%, 17.4% and 19.6%, respectively, during the 3-year period. In contrast, the size of the population in the catchment area increased by 0.6%. Overall, the rate of fungaemia was 0.51/1000 admissions, and 10.4/100 000 population, but a slight increase was observed when comparing 2004 and 2006 (9.6/100 000 inhabitants, 95% CI 8.6–10.7/100 000 vs. 11.2/100 000 inhabitants, 95% CI 10.1–12.3/100 000, respectively). The proportion of males remained constant at 56%. The median age rose slightly from 64.0 to 66.5 years (range 0–94 years); only 1.9% of the patients were aged <1 year (incidence 16.3/100 000), 4.4% were aged <20 years (incidence 2.2/100 000), 50.0% were aged >65 years (incidence 36.9/100 000), and 36.5% were aged >70 years (incidence 38.3/100 000). The median age and the proportion of isolates in each age group varied according to species (Table 2). Notably, *C. glabrata* and *C. krusei* were not recovered from any patient aged <20 years.

The overall species distribution is shown in Table 1. *Candida* spp. accounted for 98.1% of the fungal isolates, with *C. albicans* being the predominant species (overall 59.8%). However, the proportion of *C. albicans* varied considerably among the participating hospitals, from 51.1% at Hvidovre Hospital in Copenhagen to 67.6% at the hospitals in the county of North Jutland. *C. glabrata* was the second most frequent species (overall 20.5%), again with considerable variation in frequency according to hospital (11.2–33.8%) and the blood culture system used. The variation in distribution of *C. glabrata* between centres using BACTEC (16.7%, 97/580) and those using BacT/ALERT (24.4%, 135/553) was statistically significant (p 0.0015). *C. krusei* was a rare isolate (4.1%) throughout the entire study period.

Table 2. Distribution of fungal isolates according to age group

Species	No. (%) of isolates found in group aged (years)					
	<1	1–20	21–40	41–60	61–80	>80
Candida albicans	15 (71)	23 (77)	40 (62)	191 (61)	345 (58)	64 (58)
Candida dubliniensis			6 (9)	6 (2)	16 (3)	1 (1)
Candida glabrata			8 (12)	65 (21)	125 (21)	34 (31)
Candida krusei			2 (3)	18 (6)	26 (4)	1 (1)
Candida parapsilosis	4 (19)	4 (13)	4 (6)	11 (4)	21 (4)	1 (1)
Candida tropicalis				12 (4)	34 (6)	6 (5)
Candida spp.	2 (10)	2 (7)	1 (2)	5 (2)	15 (3)	2 (2)
Other fungi		1 (3)	4 (6)	3 (1)	12 (2)	1 (1)
Total	21	30	65	311	594	110

During 2004–2006, the total number of *C. albicans* isolates was stable, although the overall proportion decreased significantly, while the number of *C. glabrata*, *C. krusei* and *C. dubliniensis* isolates increased (Table S1, see Supplementary material). None of these changes was caused by a single-centre effect. Mixed infections involving two fungal species occurred in 44 (4.0%) cases, i.e., eight in 2004, 19 in 2005 and 17 in 2006 (2004 compared to 2005–2006, p 0.07). In 36 of these cases, *C. albicans* was isolated in combination with another yeast, with *C. glabrata* accounting for 29 cases. In total, 42 (95.5%) of the polyfungal infections involved at least one isolate with intrinsic decreased susceptibility to fluconazole (i.e., *C. glabrata*, *Saccharomyces cerevisiae*, *C. krusei* and *Rhodotorula* spp.).

Antifungal susceptibility testing

Susceptibility testing for amphotericin B, caspofungin, fluconazole and itraconazole was performed for 1025 (90.5%) isolates, and for voriconazole for 951 (83.9%) isolates.

MIC distributions are shown in Table 3. All but four isolates were susceptible to amphotericin B, as defined by an MIC of ≤1 mg/L (one *C. glabrata*, one *C. krusei* and two *Fusarium* spp. isolates had an MIC of 2 mg/L). For caspofungin, a uniform bell-shaped population was revealed, with MICs of ≤0.03–2 mg/L; however, three isolates had an MIC of 4 mg/L (one each of *Candida parapsilosis*, *Candida guilliermondii* and *Rhodotorula* spp.), and 12 (1.2%) isolates with an MIC ≥8 mg/L were detected (one *Trichosporon asahii*, four *Cryptococcus neoformans*, four *Fusarium* spp. and three *Candida* isolates (one *C. albicans*, one *C. dubliniensis* and one *C. parapsilosis*). Within *Candida* spp., a

Table 3. MIC ranges and MIC_{50} and MIC_{90} values (mg/L) for 1025 fungal isolates

	Amphotericin B			Caspofungin			Fluconazole			Itraconazole			Voriconazole[a]		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Candida albicans	≤0.06–1	0.25	0.5	≤0.06–1	0.25	0.5	≤0.125–>16	0.25	0.5	≤0.03–0.25	≤0.03	0.06	≤0.03–0.125	≤0.03	≤0.03
Candida dubliniensis	≤0.06–0.25	≤0.06	0.125	≤0.06–1	0.5	1	≤0.125–1	0.25	1	≤0.03–0.06	≤0.03	0.03	≤0.03	≤0.03	≤0.03
Candida glabrata	≤0.06–2	0.25	0.5	0 ≤ 0.06–2	0.5	1	0.5–>16	8	>16	≤0.03–>4	1	2	≤0.03–>4	0.25	1
Candida krusei	0.25–2	1	1	0.25–2	0.5	2	1–>16	>16	>16	≤0.03–0.5	0.25	0.5	0.06–1	0.25	1
Candida parapsilosis	≤0.06–1	0.5	1	0.25–>8	2	2	0.125–>16	1	4	≤0.03–0.125	0.06	0.125	≤0.03–0.25	≤0.03	0.06
Candida tropicalis	0.125–1	0.5	1	≤0.06–2	0.5	1	0.125–>16	1	2	≤0.03–1	0.03	0.25	≤0.03–2	≤0.03	0.25
Candida spp.	≤0.06–1	0.25	1	0.125–4	1	2	≤0.125–>16	1	>16	≤0.03–>4	0.125	1	≤0.03–2	≤0.03	0.25
Other fungi	≤0.06–2	0.5	2	0.5–>8	4	>8	1–>16	8	>16	≤0.03–>4	0.5	>4	≤0.03–4	0.5	4

[a]Voriconazole MICs were determined for 951 isolates.

tendency towards higher MICs of caspofungin was observed for non-*albicans* isolates (MIC_{50} 0.5 mg/L, range ≤0.06 to >8 mg/L) compared to *C. albicans* isolates (MIC_{50} 0.25 mg/L, range ≤0.06 to >8 mg/L) (Table 3).

The susceptibility patterns for the azoles were more complex. Thus, most *Candida* isolates were susceptible to fluconazole and itraconazole, with the exception of isolates belonging to the intrinsically less susceptible or resistant species *C. glabrata* and *C. krusei*. However, using the EUCAST breakpoints for fluconazole of S ≤2 mg/L and R >4 mg/L [19], 13 *Candida* isolates, which were not *C. glabrata* or *C. krusei*, were resistant (three *C. albicans* with MICs of 8 to >16 mg/L, one *Candida pelliculosa* with an MIC of 8 mg/L, and one each of *C. guilliermondii*, *Candida holmii*, *Candida inconspicua*, *Candida kefyr*, *Candida lusitaniae*, *C. parapsilosis*, *Candida tropicalis* and *Candida utilis*, all with MICs of ≥16 mg/L). In addition, 12 *Candida* isolates had an MIC of 4 mg/L (five *C. parapsilosis*, three *C. tropicalis*, two *C. guilliermondii*, one *C. albicans* and one *C. pelliculosa*). In total, 25 (2.2%) isolates of other *Candida* spp. had an MIC of ≥4 mg/L (Table 3). For itraconazole, 14 non-*glabrata* non-*krusei Candida* isolates had MICs of >0.125 mg/L (six *C. tropicalis*, three *C. guilliermondii*, two *C. pelliculosa* and one each of *C. albicans*, *Candida famata* and *C. holmii*). Ten of these 14 isolates were cross-resistant to fluconazole. Finally, all *C. albicans* isolates had voriconazole MICs of ≤0.125 mg/L, while 19 *C. glabrata* isolates had voriconazole MICs of >1 mg/L. Such isolates were detected with increasing frequency during the 3-year period (2004, 1/40, 3%; 2005, 6/77, 8%; 2006, 12/86, 14%; p 0.03).

Nineteen of the 21 non-*Candida* isolates were tested for antifungal susceptibilities. Nine were *S. cerevisiae* (fluconazole 4–16 mg/L, itraconazole 0.25–2 mg/L, voriconazole 0.125–0.5 mg/L), four were *Cryptococcus neoformans* (fluconazole 4–16 mg/L, itraconazole 0.06–0.5 mg/L, voriconazole 0.03–0.5 mg/L), four were *Fusarium* spp. (fluconazole >16 mg/L, itraconazole >4 mg/L, voriconazole 1–4 mg/L), one was *Rhodotorula* sp. (fluconazole 1 mg/L, itraconazole 4 mg/L, voriconazole 4 mg/L) and one was *Trichosporon* sp. (fluconazole 1 mg/L, itraconazole <0.03 mg/L, voriconazole 0.06 mg/L). Thus, overall, 29.8% of the fungal isolates tested (305 of 1025) showed decreased susceptibility to fluconazole and/or itraconazole (defined as an MIC of ≥4 mg/L and ≥0.25 mg/L, respectively). There was an increase in each year of the study, with 20.2% (65/321) in 2004, 28.2% (96/341) in 2005, and 35.3% (128/363) in 2006 (p 0.02 comparing 2004 and 2005; p 0.0001 comparing 2004 and 2006).

Consumption of antifungal agents

The total consumption of antifungal agents in Denmark is shown in Fig. 1. The total annual usage of fluconazole increased by 152% (from 13.6 to 34.4 DDD/100 000 inhabitants/day) during the 5-year period from 2001 to 2006. For the systemic azoles, 78% of usage was in the primary healthcare sector (http://www.medstat.dk/PackStatDataViewer.php).

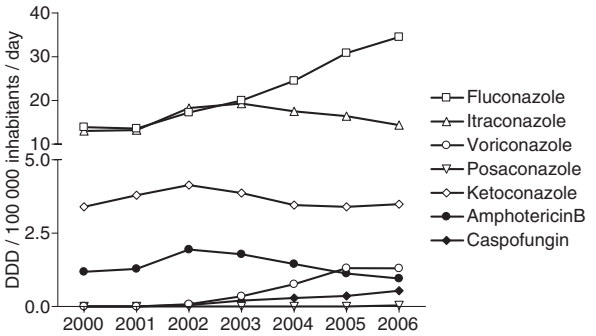

Fig. 1. Use of antifungal compounds (in daily defined doses, DDD) in Denmark between 2000 and 2006.

DISCUSSION

The first comprehensive Danish study on fungaemia, performed in 2003, revealed a notably high annual incidence of fungaemia, which was consistent with a steady increase in the numbers of cases of candidaemia since the early 1990s [1]. The surveillance programme has subsequently been extended to include two-thirds of the Danish population, with data from the last 3 years showing a further increase, despite the fact that all tertiary hospitals in Denmark were already included in the first study period.

Surveys in other Nordic countries report annual rates of candidaemia in the range of 2–4.9 episodes/100 000 inhabitants [2–4,8]. In this context, the present estimated annual rate of 10.4/100 000 inhabitants seems very high, and is also high when compared with candidaemia rates in the rest of Europe, Canada, Iowa, Atlanta and San Francisco, USA, which were in the order of 3–8/100 000 population, but is comparable to the combined rate of 10/100 000 reported in a recent survey in Connecticut and Baltimore, USA [7,9,11,12,14,20].

The reason for the high rate of fungaemia in Denmark is not clear. One hypothesis is that the use of centrally drawn blood cultures from patients in intensive care units may contribute to a high incidence of fungaemia. However, the proportion of blood cultures positive for coagulase-negative staphylococci, which are microorganisms that are frequently associated with catheter infection, was 25–30% (data not shown), i.e., comparable to proportions reported elsewhere, which suggests that this is not the sole explanation [21,22]. Applying the estimate of Wenzel and Edmond [21] that 5% (2.5–10%) of all patients admitted to hospitals will be affected by a nosocomial infection, 10% of which will be bloodstream infections, with 8% of these being caused by *Candida* spp., a total of 294 (147–587) candidaemia cases would be expected to be detected annually in the present survey, which is in close agreement with the number actually observed. However, as the number of hospital admissions in Norway is similar to that in Denmark, a similar figure for candidaemia should also be expected in Norway [23]; the discrepancy illustrates that a number of issues concerning fungaemia rates are not yet fully understood.

The highest incidences of candidaemia were observed among elderly patients, reaching an incidence of 36.9 episodes/100 000 among the population aged >65 years. With the exception of the Baltimore and Connecticut study, this is the highest incidence reported in any population-based survey [2,4,7,8,10,12,20]. A high incidence was also revealed in infants (16.3/100 000), confirming the susceptibility to candidaemia of this patient group [2,4,8]. The proportion of candidaemic patients aged >70 years was lowest for *C. parapsilosis* (26.7%), but *C. parapsilosis* caused only four of 21 cases of candidaemia in patients aged <1 year. Hence, the higher proportion of *C. parapsilosis* in younger age groups was confirmed, but without the marked over-representation among neonates reported in other studies [3,10,24–27]. The proportion of candidaemic patients aged >70 years was highest for *C. tropicalis* (50%), and was significantly higher than for *C. parapsilosis*. *C. tropicalis* is more frequent in patients with underlying haematological disease, and the increasing frequency of such disease in the elderly population may account for the over-representation of *C. tropicalis* in this age group. The percentage of patients aged >70 years was also higher for *C. glabrata* than for *C. albicans* (39.6%), which is in accord with previous studies [2,3,9,25,26,28].

Three species accounted for the continuous increase in candidaemia over the 3-year period, namely *C. glabrata*, *C. krusei* and *C. dubliniensis*. While the increase of *C. dubliniensis* may reflect, at least in part, enhanced efforts to distinguish *C. dubliniensis* from *C. albicans* during the study period, the increase of *C. glabrata* and *C. krusei* cannot be explained by changes in methodology. The total usage of fluconazole has increased considerably in Denmark during recent years, i.e., by 153% between 2001 and 2006, and it is possible that this increased consumption may have contributed to the increase in the number of isolates and percentages of these species (which have intrinsically higher MICs).

Recent reports have suggested that the choice of blood culture system may influence the recovery of *C. glabrata*, and that the BACTEC system may be inferior to the BacT/ALERT system in this respect [2,29]. The present data support this observation, as the recovery rate of *C. glabrata* was significantly lower at centres using the BACTEC system than at centres using the

BacT/ALERT system. It is therefore suggested that the mycosis medium is included at centres using the BACTEC system when a patient is at risk for candidaemia.

A low prevalence of resistance to amphotericin B and caspofungin was revealed, but unlike the first year of surveillance, in which no *Candida* isolates showed elevated caspofungin MICs, three *Candida* isolates were found to have caspofungin MICs >8 mg/L. The low level of resistance is not surprising, as amphotericin B is a broad-spectrum compound that has been used for many years without any noticeable selection of resistance. Usage of amphotericin B and (recently introduced) caspofungin is also much less than that of azoles. Reduced susceptibility to azoles was mainly demonstrated in isolates with intrinsic resistance mechanisms to azoles, i.e., *C. glabrata*, *C. krusei* and some non-*Candida* isolates. However, interestingly, the percentage of isolates that were not fully susceptible to fluconazole and/or itraconazole accounted for 35% of the total cases of fungaemia in 2006. Treatment regimens should take this high percentage of fluconazole-non-susceptible isolates into account. In this setting, the use of fluconazole would be safe if restricted to cases in which: (i) susceptibility testing has already been undertaken; (ii) susceptibility is reliably indicated by the species identification; or (iii) close surveillance of the individual patient suggests that fluconazole-resistant yeasts are a rare cause of fungaemia. This is in line with the recent recommendations by Laupland *et al.* [20], which were based on similar proportions of isolates with reduced susceptibility to fluconazole. This emphasises the need for rapid species identification and susceptibility testing, which is now facilitated by commercially available rapid identification tests for *C. glabrata* and *C. krusei.*

In conclusion, the present study revealed a remarkably high and continuously increasing incidence of fungaemia in Denmark compared to other Nordic countries and worldwide. The proportion of isolates with decreased susceptibility to fluconazole and/or itraconazole exceeded one-third. These worrying findings underline the importance of monitoring the situation and adjusting the initial choice of antifungal agents and the use of antifungal prophylaxis according to the local patterns of epidemiology and antifungal susceptibility.

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SUPPLEMENTARY MATERIAL

The following Supplementary material for this article is available online at http://www.blackwell-synergy.org.

Table S1. Species distribution according to year and centre, as registered in the semi-national fungaemia surveillance programme 2004–2006

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